

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 13, line 19 to read:

In one embodiment of the present invention, the measurement takes place in a one-step "homogeneous" system; a homogeneous system is one in which the sample is mixed with the reagent cocktail, and no separations or further transfers are required prior to readout. The enzyme or enzymes whose activity is being measured (in enzymatic activity mode) or the enzyme or enzymes released from cells (in cytotoxicity, membrane-damage, proliferation, or combined cytotoxicity/proliferation mode) are coupled in a single reaction vessel to production of ATP, NADH, or another high-energy molecule which is a substrate for a luciferase; the luciferase then produces light from the chemical energy of the high-energy molecule. The increase or decrease in the luminance signal is related to the concentration(s) of the enzyme or enzymes whose activity or activities are of interest. Taking cytotoxicity assays as an example, the reagent cocktail may be added to the cells under test before, after, or simultaneously with the potentially cytotoxic agent, depending on the kind of test being performed. If a quantitative determination of killing rate were desired, the cells could be mixed with the agent first and incubated for a fixed interval, after which the reagent cocktail would be added; this would provide an accurate picture of aggregate cell death over time. For maximum speed, reagent cocktail, cells, and the potentially cytotoxic agent could be mixed simultaneously; depending on the speed of killing, a signal could be obtained within minutes, or possibly even less than one minute. Finally, mixing the reagent cocktail with cells before addition of the potentially cytotoxic agent would allow comparison of the viability before and after treatment. These last two modes would also allow the user to follow the whole toxicity reaction in real time. A calibration standard of cells could be used to obtain absolute quantification. Note that the homogeneous nature of this aspect of the invention distinguishes it, in the case of cytotoxicity,

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from the GPL method, in which the assay reagents are not added in a single reagent mixture; instead the GPL method requires multiple transfers and incubations, first from the sample being tested to the "GP" cocktail; next, following an incubation, from the GP cocktail to the luciferase cocktail, which must also be aliquoted separately. Moreover, the GPL assay is not compatible with live cells, which must be separated by centrifugation, filtration, or another method before the first transfer. In the present invention, all constituents necessary for the assay are added in a single aliquot to the sample being tested, and there is no need to remove live cells from the supernatant.

Please amend the paragraph beginning at page 22, line 2 to read:

In one aspect, the present invention is directed toward methods of measuring cytotoxicity. In a preferred embodiment, cytotoxicity is measured in a homogeneous assay in a microplate luminometer. The luminance signal is produced by firefly luciferase acting on adenosine triphosphate (ATP), which in turn is produced by the coupled reactions of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and phosphoglycerokinase (PGK), two consecutive enzymes of the glycolytic pathway. G3PDH, a very abundant enzyme in all known cells, is measured to quantify release (and therefore cell death and/or membrane damage), while PGK, which is generally not so abundant in cells, is supplied in the reaction cocktail, along with glyceraldehyde-3-phosphate (G3P), nicotinamide adenine dinucleotide oxidized form (NAD⁺), inorganic phosphate (Pi), dithiothreitol (DTT), adenosine diphosphate (ADP), the components of the luciferase reaction, and appropriate buffers and salts (see Figure 1 for a schematic diagram of the assay, and Example 1 for additional details of the components). Essential to the invention is the fact that G3PDH is abundant in all living cells; therefore the user can be confident that the invention will be ~~using~~ useful in measuring cytotoxicity and/or proliferation of a specific cell

type without prior testing. Moreover, G3PDH is a natural component of the cells, and does not need to be introduced into the cells in any manner. This distinguishes the present invention from all methods which require prelabeling of the cells, or transfection, transformation, or other methods of introducing proteins or other molecules into the target cells in order to generate a signal in a later step.

Please amend the paragraph beginning at page 34, line 20, to read:

The 5X PKG diluent was made as follows:

3.73 g of Triethanolamine (TEA) = 25 mM

[[15]] 1.5 g NaH₂PO₄

1.295 mL 193 mM Ethylene Diamine Tetraacetic Acid (EDTA) pH 8.0

25 mg Bovine Serum Albumin (BSA) Fraction V

Titrated to pH 7.0 with concentrated HCl and made up to a final volume of 50 mL.

Please amend the paragraph beginning at page 37, line 3, to read:

The contents of each reaction vessel were aliquoted in duplicate onto a microtiter plate. A fixed amount of 0.005 mL of 1:10,000-diluted G3PDH was added in duplicate to each PGK dilution and the reactions were read for luminance. The results [[are]] showed that PGK diluted 1×10^{-4} from the purchased reagent yielded an excellent signal, although saturation was seen, which proved to be due to exhaustion of ADP. Still higher concentrations of PGK led to sublinear behavior even after the ADP concentration was adjusted (see Example 2C, Figure 7). This concentration of PGK (1×10^{-4}) was therefore selected for the optimized cocktail. However, since the level of G3PDH contamination in a different lot of PGK could be higher, it may be necessary to test each lot for this problem when in commercial production. If the

G3PDH contamination is unacceptably high, another source can be found, or the PGK enzyme can be purified away from G3PDH, or labile, irreversible inhibitors of G3PDH such as iodoacetic acid can be used to inactivate the contaminant.

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